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PRINCIPAL INVESTIGATOR: Ursula Matulonis, MD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02215

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Ursula Matulonis, MD

E-Mail:Ursula_matulonis@dfci.harvard.edu

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Dana-Farber Cancer Institute
450 Brookline Avenue, YC 1421
Boston MA 02215-5450

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14. ABSTRACT

In this project low grade serous cancer, clear cell, endometrioid (low grade and high grade),and mucinous cancers will be interrogated and subjected to somatic mutation profiling using genome sequencing and array comparative genomic hybridization (aCGH) to explore copy number alterations (CNA). To date, over 200 subjects have enrolled and pathology has identified blocks for DNA extraction. The Study remains important as nothing has been published on the molecular alternation of the different histological subtypes of ovarian cancer.

15. SUBJECT TERMS
Ovarian Cancer, histological subtypes, somatic mutations

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Table of Contents **Page**

I INTRODUCTION

Several key advancements have shaped the understanding of ovarian cancer genomics as well as exploiting and translating these discoveries into therapeutic advances for ovarian cancer patients. Although once considered a single entity, ovarian cancer can be now subdivided into different histological subtypes that have different identifiable risk factors, molecular compositions, clinical features, and, more recently, treatments. These histologies include high grade serous carcinoma (HGSC) and low grade serous carcinoma (LGSC), as well as endometrioid, clear cell, and mucinous carcinoma (1-3).

The most common histologic subtype of ovarian cancer, HGSC, has undergone the most extensive genomic investigation and is characterized by underlying deficiencies in DNA repair, presence of BRCA mutations in some tumors, and evidence homologous recombination deficiency (HRD) abnormalities in about 50% of HGSC cases (1-3). Oral poly (ADP ribose) polymerase (PARP) inhibitors exploit the HRD abnormalities of HGSC for their efficacy, leading to the United States Food and Drug Administration approval of the oral PARP inhibitor olaparib. The approval of olaparib in 2014 was the first targeted and personalized therapy for ovarian cancer, specifically for women with recurrent ovarian cancer who have a germline *BRCA* mutation.

The overarching goal of this Department of Defense (DOD) project has been to comprehensively genetically characterize ovarian cancer at our institution in order to identify targetable and actionable mutations.

II. BODY OF WORK:

Body of work:

<u>Year 1:</u>

During year 1, the following was intended to be accomplished:

1) <u>Specific aim 1 will be started:</u>
-- 75 tumor samples (formalin fixed paraffin embedded) from different histologic subtypes of ovarian cancer will be pulled from the Department of Pathology at the Brigham and Women's Hospital. These histologic subtypes will include: high grade serous, low grade serous, clear cell, high grade endometrioid, and mucinous cancer.
-- Clinical data will be extracted for each case.
-- Areas of highest percentage of tumor will be assessed by a gynecologic oncology pathology, circled and cored.
-- Somatic mutation assessment, copy number variation and analysis as well as gene expression

profiling will be started on these samples.

Progress:
Specific Aim 1 was started. Specific aim 1 is as follows:

Specific aim 1: Newly diagnosed epithelial ovarian cancer patients and active patients with recurrent cancer undergoing treatment at the DFCI will have their cancers tested for cancer gene mutations as well as copy number alterations. This project seeks to identify the molecular and genetic abnormalities of ovarian cancer in order to better choose and select rationale therapies for women with ovarian cancer.

Protocol 11-104, which is an Institutional Review Board (IRB)-approved Dana-Farber/Harvard Cancer Center (DF/HCC) research protocol, enrolled patients and subjected cancer samples to a test called OncoPanel (4). Clinical research staff from the Dana-Farber Cancer Institute (DFCI) Gynecologic Oncology program supported by this grant consented patients to 11-104. OncoPanel is a cancer genomic assay that detects somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. DNA was isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer (4). This genetic profiling is performed within the Department of Pathology at the Brigham and Women's Hospital in Boston MA. In addition, the results of OncoPanel are resulted and available for viewing in a restricted website that the patient's attending physician can access. This is a research test and it is not billed to patients. Through this protocol 11-104, clinical data extraction was also started for cases.

The statistician on this grant, William Barry, Ph.D, assisted in all aspects of statistical analysis of this project.

Year 2:

During year 2, the following will be accomplished:

1) Specific aim 1 will be completed.

2) Specific aim 2 will be started:
-- Our analysis will begin by looking within each genomic data type to search for additional common robust subtypes and to explore whether any correlation exists between the OncoMap genomic alterations or copy number alterations and the expression-based subtypes.

-- Additional subtypes will be looked for defined by molecular profiles by extending our analysis beyond single mutational events and looking for common-pathway mutations that might indicate commonly disrupted processes within subsets of HGSC patients.

-- Dr. Quakenbush and his group will then investigate clinical correlation between outcome and results of the OncoMap, aCGH, and expression profiling.

Progress:
Specific aim 1 was completed and Specific aim 2 was started.

Specific aim 1: This task was completed and over 1000 samples of ovarian cancer have been identified and genomically characterized.

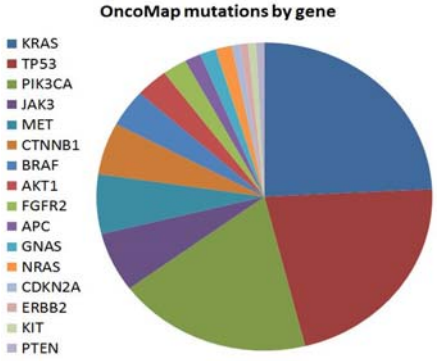
Since this grant's inception, more than 1000 patients with ovarian cancer have been enrolled into 11-104 and consented by our data managers in the Gynecologic Oncology program. Through a DF/HCC IRB request placed by Dr. Matulonis for 11-104 ovarian cancer data, data retrieval and analysis was initiated in 2015 with the assistance of William Barry, PhD, the statistician on this project. This project included Elizabeth Stover, M.D., Ph.D, currently an Instructor of Medicine at Harvard Medical School who joined this project in April 2015 and has been on faculty in the DFCI Gynecologic Oncology program since July 2016.

This grant has led to several publications and has been significantly instrumental for our program's development of precision medicine for our patients with ovarian cancer which are listed in Section IV (REPORTABLE OUTCOMES). In addition, the de-identified genomic information generated from this grant can be accessed other research investigators at DFCI for collaborative studies across cancer types; these investigators must submit a formal request to access this data to the DF/HCC IRB to access this information and also receive permission from Dr. Matulonis who is the gynecologic oncology user committee lead.

Accessible clinical information as per DF/HCC IRB approved protocol 11-104 includes ovarian cancder stage, histology, type of initial treatment (upfront surgical debulking versus neoadjuvant therapy), progression free survival, time to first recurrence, and responses to administered treatments will provide critical outcome data for current and future abstracts and projects.

Specific aim 2:
We have started specific aim 2 and 302 patients were tested for hotspot mutations in 41 genes by OncoMap. Most tumors were primary tumors, with 8% metastatic. Histologic types included 89% adenocarcinoma and its subtypes, 4% sex cord stromal, 2% borderline, and ~1.5% each carcinosarcoma, germ cell, and other. 128 mutations were found in 102 patients; most patients had 1 mutation (see below Figure 1).

Figure 1:



OncoMap genes mutated in ovarian cancer

Distribution by

Gene	Mutations	N
AKT1	E17K	4
BRAF	V600E,K	5
CTNNB1	S33C,F	2
	S37C,F	4
FGFR2	C382R	2
JAK3	P132T	2
	V722I	6
KRAS	G12A,C,D,V	26
	G13C,D	2
	Q61H,K	2
MET	T1010I	8
PIK3CA	E542K	5
	E545K	2
	H1047L,R	12
	R88Q	2
TP53	R175H	7
	R248Q,W	11
	R273C,H	8

OncoMap mutations identified two or more times in the OncoMap cohort of 128 mutations (N=number of mutations). The OncoMap platform is designed to genotype known hotspot mutations, so all have biologic relevance. Several mutations are potentially targetable with small molecule inhibitors including BRAF V600E, MET T1010I, and PIK3CA mutations. Overall, approximately 1/4 of patients exhibit a potentially targetable alteration using this panel of oncogenic mutations.

Mutations found in specific ovarian cancer histologies:

We also identified specific mutations for specific histologic subtypes:
Table 1

Histology	Most frequently altered genes
High grade serous	TP53, BRCA1/2, ARID1A/B, KMT2D, APC, CREBBP, PRKDC
Low grade serous	KRAS
Endometrioid	ARID1A/B, PTEN, BRCA1/2, APC, KMT2D, ATRX, NF1, CTNNB1, MTOR, PIK3CA
Clear Cell	ARID1A/B, PIK3CA, BRCA2, ATM, SETD2, ATRX, MTOR, PIK3R1
Mucinous	KRAS, TP53, CDKN2A, SMAD4, TERT
Carcinosarcoma	TP53, RB1, APC
Granulasa cell	ATM, KMT2D, EP300, FANCD2
Sertoli Leydig cell	DICER1

Copy number variants: In addition, 219 patients were analyzed for copy-number variations (CNV) in OncoPanel genes. >12,000 total CNV were reported in the cohort (Figure 2). Single-copy deletions (n=5558) and copy-number gains (low amplification) (n=6635) were numerous. 188 highly amplified genes were reported from 64 patients, and 130 2-copy deletions from 33 patients, largely high-grade serous carcinoma and carcinosarcoma.

Figure 2:

Frequent CNV events

Most frequent highly-amplified and 2-copy deleted genes

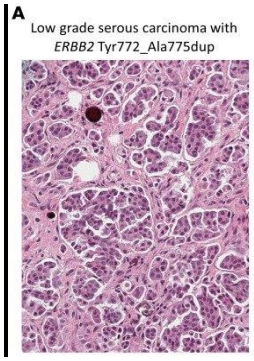
Highly amplified	N
MECOM	11
CCNE1	9
AKT2	7
FGFR1	7
PIK3CA	7
PRKCI	7
MYC	6
ERBB2	5
KRAS	5

2-copy deletion	N
STK11	5
CDKN2B	4
GNA11	4
RB1	4
FGFR3	3
FLCN	3
MAP3K1	3
NF1	3
PHOX2B	3
PIK3R1	3
PTEN	3

Specific actionable mutations have been found:

Sholl et al (Section IV: REPORTABLE OUTCOMES) Rare activating ERBB2mutations in ovarian cancer. A patient with a low grade serous cancer, heavily pretreated for her recurrent cancer, had her recurrent tumor analyzed and identified was an *ERBB2* Tyr772_Ala775dup mutation (Figure 3, from Sholl et al). Demonstrating this mutation resulted in the use of trastuzumab and navelbine with anti-cancer response radiographically and symptomatic improvement (less pain) for over 2 years.

Figure 3: Histology from a 48 yo patient with low grade serous carcinoma



III. KEY RESEARCH ACCOMPLISHMENTS:

1) Genotyping and NGS of cancer-associated genes in formalin-fixed tissue from ovarian tumors are feasible and can identify potentially clinically actionable alterations.

2) Known and novel single nucleotide variants and copy-number variations were identified in each histologic subtype.

IV. REPORTABLE OUTCOMES

Ritterhouse L, Nowak J , Strickland K, Garcia E, Jia Y, Lindeman N, MacConaill L, Konstantinopoulos P, Liu J, Berkowitz R, Nucci M, Crum C, Matulonis U, Sholl L, Howitt B. Morphologic Correlates of Molecular Alterations in Extra-uterine Müllerian Carcinomas, Mod Pathol. 2016 Aug;29(8):893-903

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V. CONCLUSIONS:

1) Both copy number and mutational data are now available for accessing on over 1000 ovarian cancer patients as of February 2016. Importantly, this genomic information is available to other research investigators at DFCI for collaborative studies who are IRB approved to access this information and provided in a de-identified manner.

2) For the ovarian cancer cohort, this data has now been accessed and several abstracts and manuscripts are in process. Dr. Elizabeth Stover, formerly a fellow at DFCI, has joined Dr. Matulonis' group at DFCI and has joined our faculty in July 2016; this work is a crucial part of her academic development.

3) Identification of important aberrant pathways found in ovarian cancer has led to new treatment strategies for ovarian cancer treatments such as HER2 mutations.

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